ABSORPTION OF AMMONIUM AND NITRATE BY MYCORRHIZAL AND NON-MYCORRHIZAL ROOTS OF PINE

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SUMMARY

Studies investigated the absorption of NH_4^+ and NO_3^- by mycorrhizal and non-mycorrhizal roots of Pinus contorta Dougl., P. ponderosa Laws. and P. taeda L. Fungal symbionts included Pisolithus tinctorius (Pers.) Coker and Couch and Thelephora terrestris Ehrh. ex Fr.

Typical ion absorption kinetics were observed with an initial rapid uptake followed by a leveling-off of the absorption curve indicating absorption site saturation phenomena. Time required to attain saturation varied with the species, but rapid increases in ion absorption were minimized after 4-8 hr incubation.

Saturation levels for non-mycorrhizal roots were significantly different in both NH_4^+ and NO_3^- uptake. P. ponderosa exhibited the greatest absorption of both ions while the greatest differential in absorption ratio (NH_4^+/NO_3^-) was found with P. taeda. Such results indicate possible ecological adaptability inherent in the physiology of the species.

Mycorrhizae exhibited greater absorption of both ions than non-mycorrhizal roots in all cases. Differences between fungal species were not great in either NH_4^+ or NO_3^- absorption. However, P. tinctorius mycorrhizae exhibited a somewhat more "efficient" uptake system. The absorption ratio was lower for mycorrhizae compared to the non-mycorrhizal roots suggesting that the fungi most likely possess the NO_3^- reducing system which becomes operative upon exposure to NO_3^-.

Keywords: Mycorrhizae, ammonium, nitrate, ion uptake

INTRODUCTION

It is generally recognized that mycorrhizae play an important role in seedling growth and survival (Zak 1964). Among the benefits afforded the seedling by the symbiotic association, mycorrhizae aid in the absorption of both inorganic and organic nutrients (Hatch 1937, Marx 1972, Slankis 1974). Historically

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This attribute has been commonly used to explain the beneficial role of mycorrhizae in seedling growth (Zak 1964).

A majority of the studies concerned with a mycorrhizal response have centered around the macronutrients nitrogen, phosphorus and potassium, though evidence has periodically appeared in the literature in reference to the absorption of other elements. However, even nitrogen has not been comprehensively studied in terms of the comparative absorption kinetics of mycorrhizae and non-mycorrhizal short roots.

Carrodus (1966) found that excised mycorrhizal roots of *Fagus sylvatica* readily absorbed NH_4^+ from an NH_4Cl solution, but not NO_3^-. In addition, the absorption of NH_4^+ could be associated with normal metabolic processes involved in respiration and oxidative phosphorylation. This relationship is supported by stimulation of NH_4^+ uptake by the addition of exogenously applied sugars (Carrodus 1966).

Much emphasis has been placed on the utilization of various nitrogen forms by the fungal symbiont comprising the mycorrhiza. In the case of ectomycorrhizae, this is probably due to the morphology of the infected root. Lundeberg (1970) screened 27 mycorrhizal fungi in pure culture for their performance with NO_3^- and NH_4^+ sources. Results varied both between and within species (between isolates). Most species performed best with NH_4^+ as a source of nitrogen while a few isolates grew equally well with either NO_3^- or NH_4^+. No isolates tested exhibited a marked preference for NO_3^-. In similar pure culture studies, Norkrans (1950) found that all of the mycorrhiza-forming *Tricholoma* species tested were able to utilize NH_4^+, but NO_3^- only poorly.

Those fungal species able to readily utilize NO_3^- must undoubtedly possess nitrate reductase. As reported by Trappe (1967), nitrate reductase was found in sporocarp tissue in two of eight ectomycorrhizal fungal species tested. More recent work (I. Ho and J. M. Trappe, personal communication) has shown nitrate reductase activity to be detectable in the mycelium of cultures of seven tested ectomycorrhizal fungi.

The studies reported in this paper were undertaken to investigate the absorption of NH_4^+ and NO_3^- by mycorrhizal and non-mycorrhizal short roots of *Pinus contorta* Dougl., *P. ponderosa* Laws. and *P. taeda* L. Fungal symbionts included *Pisolithus tinctorius* (Pers.) Coker and Couch and *Thelephora terrestris* Ehrh. ex Fr.

EXPERIMENTAL METHODS

Seed of each pine species (known source) were surface-sterilized in 0.8% $NaHClO_4$ and germinated on 1.6% (w/v) corn meal agar. Seedlings, approximately one week old, were planted in axenic culture systems consisting of a vermiculite-peat substrate (15:0.1, v/v) watered with a modified Melin-Norkrans nutrient solution (Ahrens and Reid 1973, Bryan and Zak 1961, Hacskaylo 1953). After 30 days, randomly selected systems were inoculated with 30 ml of a liquid suspension of pure culture grown mycorrhizal fungus. Systems were maintained in a greenhouse waterbath under a 16-hour photoperiod and substrate temperatures of 18-24°C.

After 12 months, the containers were disassembled, and the root systems were washed with distilled water and destructively sampled by excising mycorrhizal short roots from inoculated seedlings and non-mycorrhizal short roots from non-inoculated seedlings. Care was taken to remove only short roots so as not to allow mother root[2/] material in the analyses.

Excised roots of each species were pooled together and rinsed in distilled water. Approximately 50 root tips/treatment were placed in 0.25X modified Hoagland's nutrient solution containing 50 mM nitrogen as either NH_4^+ or NO_3^- (Table I). Each treatment was replicated three times. Solution pH was monitored periodically and maintained at 6.0 ± 0.2 pH units by adjustment with 0.1 N HCl or NaOH. Treatment solutions were continuously shaken at 23°C, and incubation was allowed to continue for 24 hours.

One-milliliter solution samples were removed periodically and analyzed for NH_4^+-N and NO_3^--N. The NH_4^+-N determination involved direct nesslerization while NO_3^--N was derived from the modified cadmium reduction reaction involving gentistic acid (Strobbe 1972). Concentration of these ions was assessed colorimetrically.

Net ion absorption by root material was obtained by the difference in initial ion concentration and nitrogen concentration in the incubation solution at sampling time. Absorption is reported as micromoles of NH_4^+ or NO_3^- per milligram dry weight of tissue.

RESULTS

Typical ion absorption kinetics were observed with an initial rapid uptake followed by a leveling-off of the absorption curve indicating absorption site saturation phenomena (Epstein 1972) (Figures 1-6). Time required to attain saturation varied with the species, but rapid increases in ion absorption were minimized after approximately 4-8 hours incubation.

NO_3^- absorption by both mycorrhizae and non-mycorrhizal roots leveled off near termination of the experiment for all three tree species. NH_4^+ absorption remained at an increasing rate after 24 hours with *Pinus ponderosa* and *P. taeda*. Lesser increases were found with *P. contorta*.

Saturation levels for non-mycorrhizal roots were significantly different in both NH_4^+ and NO_3^- uptake (Figures 1-6). NH_4^+ was absorbed to a greater extent as indicated by the NH_4^+/NO_3^- absorption ratio (Table II). Ratio values for all three species were greater than 1.0 with the greatest differences occurring with *P. taeda* and *P. contorta*. Lower values were exhibited by *P. ponderosa* with a general increase in absorption ratio over time.

Of non-mycorrhizal roots, *P. ponderosa* absorbed the greatest amounts of both NH_4^+ and NO_3^- after 24 hours, while *P. taeda* and *P. contorta* took up lesser quantities of these ions in decreasing order (Figures 1-6).

Mycorrhizae exhibited greater absorption of both NH_4^+ and NO_3^- than did non-mycorrhizal roots in all cases (Figures 1-6). This pattern generally occurred throughout the period of uptake with few exceptions. In addition, NH_4^+ was absorbed to a greater extent than NO_3^- with the absorption ratios occurring in a more limited range as compared to non-mycorrhizal roots (Table II). The absorption ratios were lower for mycorrhizae than for non-mycorrhizal short roots.

Differences between fungal species comprising the mycorrhiza were not great in either NH_4^+ or NO_3^- absorption (Figures 1-6). However, *Pisolithus tinctorius*

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[2] Mother roots refer to elongated higher-order laterals from which short roots originate.

mycorrhizae of all three tree species generally showed a somewhat greater uptake of nitrogen as compared to mycorrhizae formed by Thelephora terrestris.

DISCUSSION

NH_4^+ was absorbed to a greater extent than NO_3^- in all treatments investigated. Nitrification is usually very low in acid forest soils (Bowen 1973). Therefore, NH_4^+-N is typically the most abundant and readily available nitrogen source in these soils (Carrodus 1966) and would be expected to be the preferred source of nitrogen. However, most higher plants can utilize both NH_4^+ and NO_3^- (Wollum and Davey 1975) as exemplified by the pine species in this study. The preference for NH_4^+ is not unexpected since this ion shortcuts the processes involved in nitrogen metabolism. It should be noted that the seedlings used in this study were grown with a nutrient solution containing NH_4^+ as the sole inorganic nitrogen source and would be assumed to perform better on this nitrogenous species. However, the lag period required for nitrate reductase induction often reported was not evident in these experiments (see Figures 1-6). Therefore, the differences between NH_4^+ and NO_3^- appear to be a preference for NH_4^+ and not a result of a pretreatment effect.

Mycorrhizae exhibited greater absorption of both ions as compared to non-mycorrhizal roots. This may be attributed to an increased efficiency of nutrient absorption on the part of mycorrhizae (Smith 1974). Increased ion absorption can be related to either increased rates of uptake or increased surface area for absorption. Both factors are probably involved in the enhanced absorption capacity of mycorrhizae (Smith 1974).

The metabolism of the root is also important in nutrient absorption. The metabolic activity of the mycorrhiza exceeds that of the non-mycorrhizal root as evidenced by respiration rates (Kramer and Hodgson 1954, Meyer 1973, Mikola 1967, Routien and Dawson 1943, Schweers and Meyer 1970). Thus, it would be expected that ion absorption would be greater for a mycorrhiza than for a non-mycorrhizal short root if metabolic activity provides the driving force for ion uptake, especially nitrogenous species. Such a relationship is supported by the previously mentioned findings of Carrodus (1966). However, in beech mycorrhizae, Harley (1964) and Carrodus (1967) have shown a stimulation effect of NH_4^+ on dark CO_2 fixation via α-keto acid removal through amination. Therefore, a "cause-effect" relationship between metabolism and ion absorption may be reversible.

A strict comparison of absorption ratio values between tree and fungal species treatments is not valid since ion absorption was based on a dry biomass determination and not on surface area considerations. Yet generalizations can be made, and within each tree species with or without the fungus, comparisons concerning NH_4^+ and NO_3^- are valid. Pinus taeda shows a definite preference for NH_4^+ in both mycorrhizae and non-mycorrhizal roots. In contrast, P. ponderosa generally exhibits the least differential in absorption ratio even though it absorbed the greatest amounts of NH_4^+ and NO_3^-. It is interesting that neither Pisolithus tinctorius nor Thelephora terrestris greatly altered the pattern of absorption ratio values; i.e., for non-mycorrhizal roots the ratios were in the order of: Pinus taeda > P. ponderosa, P. contorta; for mycorrhizae with Pisolithus tinctorius ratios were as: Pinus taeda > P. ponderosa, P. contorta; for mycorrhizae with T. terrestris ratios were as: P. taeda, P. ponderosa > P. contorta. This indicates that the host

Table I. Composition of nutrient media for the study of NH_4^+ and NO_3^- absorption by mycorrhizae and non-mycorrhizal short roots of selected pine species.

Compound	Amount (milliliters)[a/]
KH_2PO_4	0.1
K_2CO_3	0.25
$CaCO_3$	0.5
$MgSO_4 \cdot 7H_2O$	0.2
micronutrients[b/]	0.1
sequestrene 330[c/]	0.021

[a/] Amount of a one molar solution of the compound of interest per 400 milliliters of solution; not applicable to micronutrient and iron solutions.

[b/] Includes H_3BO_3 (2.86g), $MnCl_2 \cdot 4H_2O$ (1.81g), $ZnSO_4 \cdot 7H_2O$ (0.22g), $CuSO_4 \cdot 5H_2O$ (0.08g), $H_2MoO_4 \cdot H_2O$ or $MoO_3 \cdot H_2O$ (0.02g); compounds dissolved in one liter of distilled water; 0.1 ml of solution used in final solution.

[c/] Concentration equal to 24g/l; 0.021 ml of solution used in final solution.

ble II. NH_4^+/NO_3^- Absorption Ratios of Mycorrhizal and Non-Mycorrhizal Short Roots of Three Conifer Species

	Pinus ponderosa			*Pinus contorta*			*Pinus taeda*		
me r)	Non-Mycorrhizal	Mycorrhizal P.t.	Mycorrhizal T.t.	Non-Mycorrhizal	Mycorrhizal P.t.	Mycorrhizal T.t.	Non-Mycorrhizal	Mycorrhizal P.t.	Mycorrhizal T.t.
5	1.3	1.3	1.3	2.6	1.9	1.7	8.5	3.2	1.8
5	1.3	1.2	1.3	1.1	1.0	1.1	2.8	1.9	1.9
	1.5	1.4	1.5	1.6	1.5	1.7	1.8	1.6	1.7
	1.6	1.4	1.5	1.7	1.6	1.7	2.3	1.7	1.8
	1.4	1.3	1.4	1.8	1.7	1.8	1.5	1.3	1.5
	1.9	1.7	1.9	1.9	1.7	1.8	2.2	1.8	1.9

cised roots incubated in 50 mM of NH_4^+ or NO_3^- for 24 hr at pH 6.0 ± 0.2.

t. = Mycorrhizae formed by *Pisolithus tinctorius*.

t. = Mycorrhizae formed by *Thelephora terrestris*.

Figure 1. *Pinus ponderosa* with *Pisolithus tinctorius*

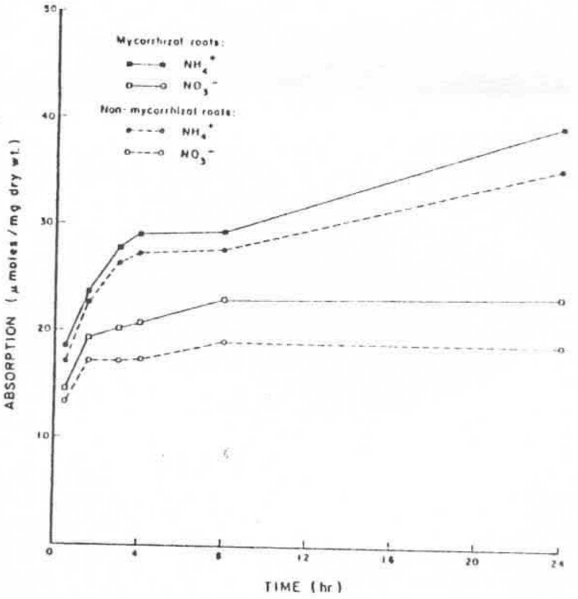


Figure 2. *Pinus ponderosa* with *Thelephora terrestris*

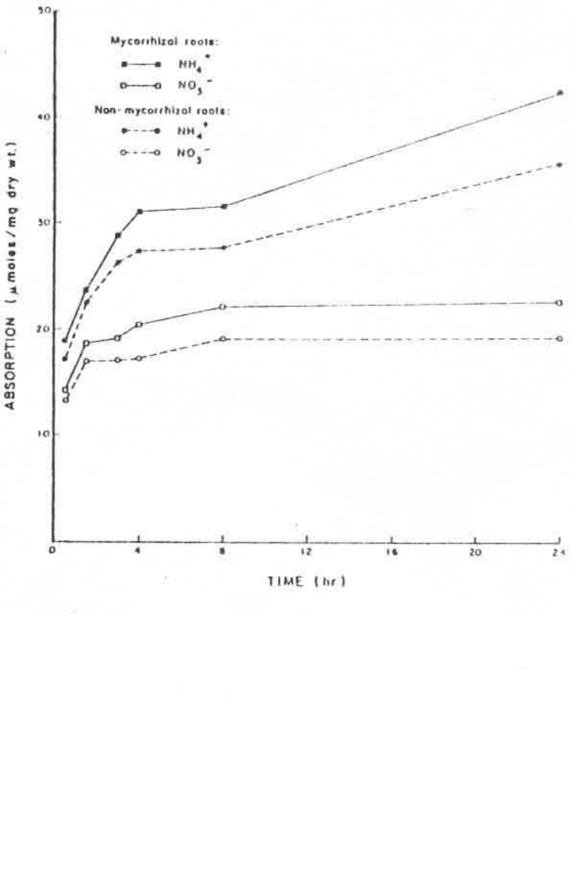


Figure 3. *Pinus taeda* with *Pisolithus tinctorius*

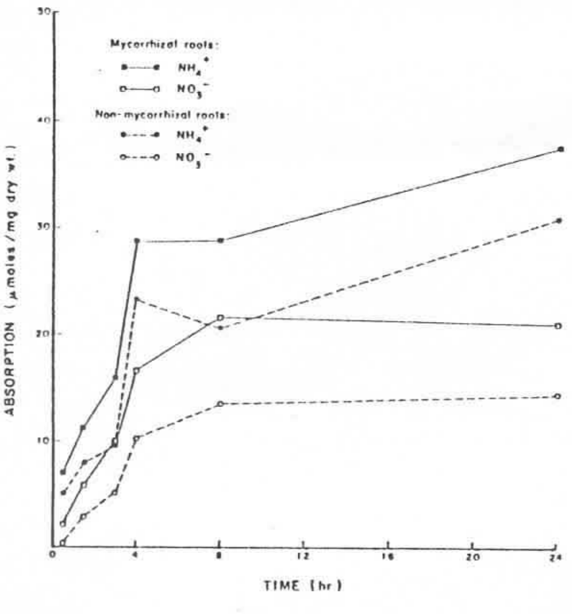


Figure 4. *Pinus taeda* with *Thelephora terrestris*

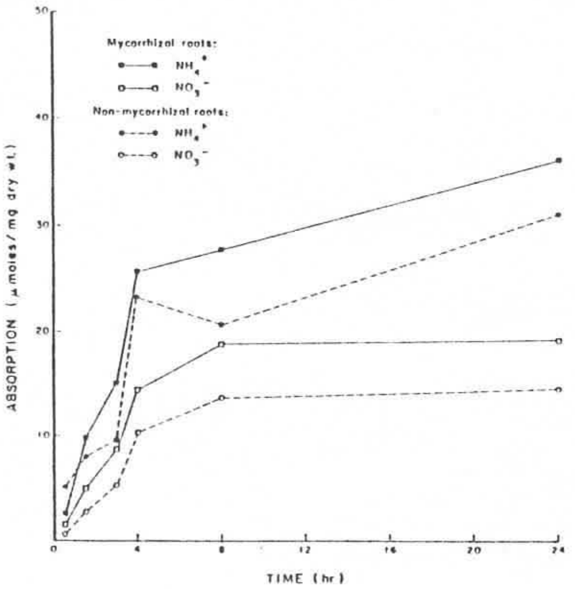


Figure 5. *Pinus contorta* with *Pisolithus tinctorius*

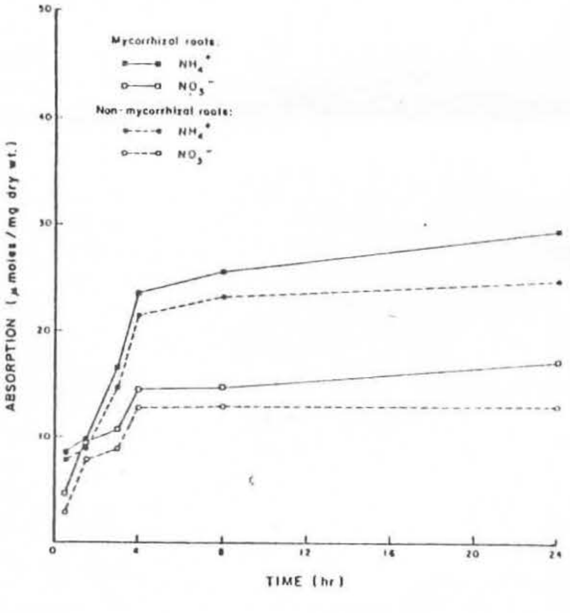
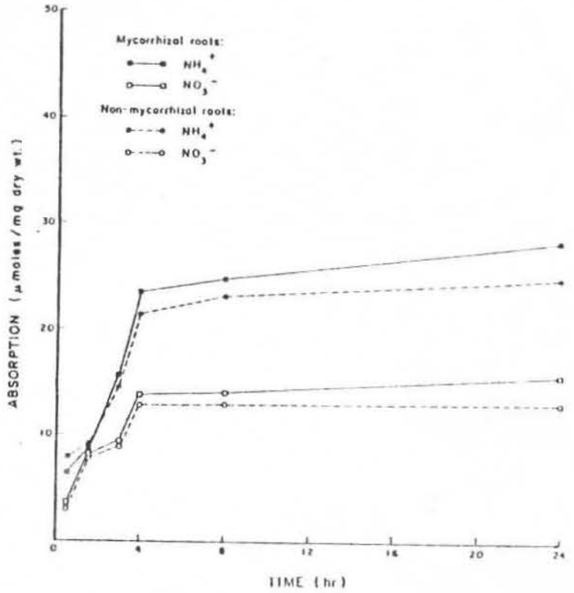


Figure 6. *Pinus contorta* with *Thelephora terrestris*



species acts in relatively the same capacity regardless of the associated fungal symbiont. However, the fungus does reduce the absorption ratio for mycorrhizae, thus suggesting that it most likely possesses the NO_3^- reducing system in the symbiotic association which becomes operative upon exposure to NO_3^-.

The results indicate possible ecological adaptability inherent in the physiology of the tree species investigated. Non-mycorrhizal roots as well as mycorrhizae of all three species possess the ability to absorb both NH_4^+ and NO_3^-, a characteristic advantageous to the organism regardless of the inorganic nitrogen form available in the soil. Such information concerning NO_3^- absorption becomes especially significant in regards to forest situations where NO_3^--N becomes important; e.g. forest nurseries (Bowen 1973).

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